Claims

1. Apparatus for electroporation comprising a wave generator, a biochip containing an array of microelectrodes and a control system that permits to transfer the signal to a pre-selected single microelectrode of the biochip.
2. Apparatus according to claim 1 characterised in that the control system consists of a personal computer equipped with a software program capable of designing various waveform signals and a switching system controlling the wave generator output.
3. Apparatus according to claims 1 and 2 characterised in that the biochip comprises an array of microelectrodes of a size comparable to the cell to be electroporated and each of said microelectrode being driven separately from the others allowing very precise and punctual control of the electroporation process.
4. Biochip comprising an array of microelectrodes comprised on a suitable insulating layer mounted on a solid substrate; means to electrically connect said microelectrodes to a switching system; a cell culture chamber where the cells can be grown and adhere in contact with said array of microelectrodes on a surface formed by said insulating layer containing said array of microelectrodes on said solid substrate.
5. Biochip according to claim 4 comprising a semiconductor substrate as solid substrate covered with an insulating layer (27) comprising an array of individually driven microelectrodes (20) of a size comparable to the cell to be electroporated, and mounting a cell culture chamber (24) with an opening (26) mounted, in turn, on a support (21) made of dielectric material, said microelectrodes (20) being electrically connected via conductive traces (28) to conductive pads (29) electrically connected, in turn, to a couple of external parallel connectors (22) through wire bonding (23) covered by the outer portion of the cell culture chamber (24) encircling the opening (26), being said cell culture chamber (24) with the opening (26) mounted over the top of the said semiconductor substrate covered with an insulating layer (27), both attached on the dielectric support (21).
6. Biochip according to claim 5 comprising two further electrodes (25) integrated

in the semiconductor substrate covered with an insulating layer (27), and acting as ground reference.

7. Biochip according to claim 5 wherein the semiconductor substrate covered with an insulating layer (27) is a silicon substrate covered with a insulating layer preferentially of SiO_2.
8. Biochip according to claim 4 and 5 wherein these solid substrates are transparent.
9. Biochip according to claim 5 wherein the dielectric support is vetronite, glass or ceramic.
10. Biochip according to claim 5 wherein the microelectrodes of the array (20) have a size with a surface of at least ten per cent of the total cell membrane and preferably a diameter ranging from 1 µm to 50 µm.
11. Biochip according to claims 4 - 10 wherein the microelectrodes are of conductive or capacitive type.
12. Microelectrodes according to claim 11 consisting of conductive microelectrodes obtained over a silicon substrate (31) covered with a insulating layer preferentially of SiO_2 (32), said microelectrodes and their connecting traces (38) being made by a "sandwich" of two titanium nitride (TiN) layers (33) and an aluminium layer (34), covered with a gold layer (37) on their active surface.
13. Microelectrodes according to claim 11 wherein said microelectrodes are realised using Metal Oxide Semiconductor (MOS) technology.
14. Microelectrodes according to claim 13 consisting of a silicon p-type substrate (40) in which two n-doped regions, drain (41) and source (42), are implanted with conventional microelectronic techniques, the gate (43) of these electrodes being realised in n+ doped polysilicon and is common to all devices in a row (word line) the drain (41) of all devices in a column being connected together by using a metal contact plug and a metal line (44), the source (42) of the transistor being connected via a metal (usually tungsten) plug (46) to a gold layer (47) which acts as the active electrode.
15. Microelectrodes according to claim 11 consisting of a capacitive microelectrode obtained with an insulating substrate (60) a metal (61) and a thin insulating layer (64) said microelectrodes being separated by insulating material (62) and

covered in non exposed areas by a passivation layer (63).

16. Method of electroporation characterised in that an apparatus according to claims 1 – 3 is used.

17. Method according to claim 16 characterised in that performs electroporation to at least a single adhering cell.

18. Method according to claim 17 characterised in that said apparatus comprises a biochip according to claims 4 - 10.

19. Method according to claims 16 - 18 characterised in that said biochip comprises microelectrodes according to claims 11 – 15.

20. Method according to claims 16 - 19 characterised in that the wave generator sends to the electrodes trains of pulses of various amplitude and duration.

21. Method according to claims 16 – 20 characterised in that the wave generator sends to the microelectrodes five trains of 25 pulses (1 ms duration) repeated at a time interval of 500 ms.

22. Method according to claims 16 – 20 characterised in that a trains of triangular voltages consisting of 10 pulses are applied to the electrodes the interval between one train and another being of 5 s.

23. Method according to any of preceding claims characterised in that it comprises substantially the following steps:

- cultivate cells since the adhering stage is reached
- add in the culture medium at least one compound to be electroporated in at least one single cell of the said cells
- selected at least one single cell and at least one microelectrode on which said selected single cell is adherent
- generate at least one electric signal suitable to electroporate said at least one single cell with said at least one compound to be electroporated and drive said electric signal to the said one microelectrode on which said selected single cell is adherent.

24. Electroporated cells characterised in that they are obtained with method according claims 16 - 23.

25. Elecroporated cells according to claim 24 wherein the electroporated agents are drugs, genetic constructs and proteins.